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Efficient Induction of Minor Histocompatibility Antigen HA-1–Specific Cytotoxic T-Cells Using Dendritic Cells Retrovirally Transduced with HA-1–Coding cDNA

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ABSTRACT

Cytotoxic T-cells (CTLs) specific for the hematopoietic system–restricted minor histocompatibility antigen (mHag) HA-1 efficiently lyse HA-1–positive leukemic cells without affecting nonhematopoietic cells. HA-1–specific CTLs are thus potential tools for adoptive immunotherapy of relapsed leukemia after HLA-matched–HA-1–mismatched stem cell transplantation (SCT). In vitro generation of HA-1–specific CTLs from SC donors is possible using dendritic cells (DCs) pulsed with synthetic HA-1 peptide as stimulator cells. However, this approach requires at least 6 weeks of in vitro culturing under GMP (good manufacturing practice) conditions. Our data show that in vitro induction of HA-1–specific CTLs is more rapid with the use of DCs that are retrovirally transduced with the HA-1 complementary DNA. Retrovirally transduced DCs showed functional and long-term stable expression of the HA-1 CTL epitope in primary CTL cultures. In 4 SC donors, HA-1–transduced DCs induced HA-1– specific CTLs in 14 to 21 days. The in vitro–generated CTL lines contained 6% to 9% T-cells that stained brightly with tetrameric HLA-A2/HA-1 peptide complexes (HA-1^{A2} tetramer) and showed significant lysis of HA-1^{+} leukemic cells. The CTL induction procedure using peptide-pulsed DCs was less effective and required 28 to 35 days of T-cell culture. Thus, sustained presentation of mHag HA-1 by retrovirally transduced DCs facilitates the in vitro induction of HA-1–specific CTLs.

KEY WORDS

Minor histocompatibility antigen HA-1 • Cytotoxic T-cells • Dendritic cells • Antigen presentation • Retroviral gene transfer • Immunotherapy

INTRODUCTION

Donor-derived cytotoxic T-cells (CTLs) directed at recipients' minor histocompatibility antigens (mHags) are important effector cells in the graft-versus-leukemia effect after HLA-matched–mHag-mismatched stem cell transplantation (SCT) [1-4]. Although several mHags are ubiquitously expressed, the expression of mHags HA-1 and HA-2 is restricted to the cells of hematopoietic lineage [5-8]. HLA-A2–restricted HA-1/HA-2–specific CTLs effectively lyse hematopoietic cells including leukemic cells and their clonogenic precursors but do not affect nonhematopoietic cells such as skin fibroblasts, keratinocytes, and epithelial and endothelial cells [5]. Thus, after an HLA-matched–mHag–HA-1/HA-2–mismatched SCT, in vitro generated HA-1/HA-2–specific CTLs from the SC donors may serve as therapeutic tools to induce the graft-versus-leukemia effect with a low risk of graft-versus-host disease (GVHD). Previously, we and others demonstrated the in vitro generation of HA-1/HA-2–specific CTLs from healthy SC donors using dendritic cells (DCs) pulsed with HA-1/HA-2 peptides as antigen presenting cells (APCs) [9-11]. This procedure, however, requires at least 6 weeks of in vitro culture. Moreover, we observed that induction of HA-1–specific CTLs from some SC donors was inefficient under GMP (good manufacturing practice) conditions (unpublished observations). Therefore, we investigated an alternative strategy for the in vitro induction of HA-1 CTLs.

In recent studies, DCs were genetically modified to endogenously express the tumor-associated antigens (TAAs) for the induction of tumor-specific immunity [12-18]. Constitutive expression of the TAAs in genetically modified DCs may not only result in effective antigen presentation to CTLs but may also allow recognition of the tumor antigen by HLA class II–restricted helper T-cells that provide help for the generation and maintenance of CTLs [19-21].

In this study, we analyzed the efficacy of the generation of HA-1–specific CTLs with DCs that are genetically engineered to express the HA-1 CTL epitope. A 358–base pair (bp) complementary DNA (cDNA) containing the genetic information for the HA-1 CTL epitope was introduced to $CD34^+$ DC progenitors using a retroviral delivery system. $CD34^+$ cells were differentiated into DCs by culturing with appropriate cytokines and were used to induce HA-1–specific CTLs from 6 $HLA\text{-}A2^+$ $HA\text{-}1^-$ SC donors. In 2 of these donors, HA-1–specific CTLs were induced in parallel with peptide-pulsed DCs to compare the efficacy of both CTL induction systems.

MATERIALS AND METHODS

Construction of the Retroviral Vector Containing the HA-1–Coding cDNA

The 358-bp cDNA encoding the HA-1 CTL epitope VLHDDLLEA was inserted into the pLZRS-polylinker-IRES-GFP retroviral vector. This vector contains the puromycin resistance gene to allow the selection of packaging cells that episomally express the retroviral vector. In addition, the vector contains the green fluorescent protein (GFP) as a marker gene and an internal ribosomal entry site (IRES) between the marker gene and the gene of interest, which ensures independent coexpression of both transgenes [22]. The HA-1–encoding cDNA was cleaved from the pCDNA–HA-1 plasmid as an XbaI/BamHI fragment, ligated into the pBluescript II SK^+, excised from the latter vector as an EcoRI/NotI fragment, and cloned into the EcoRI/NotI sites of the polylinker of the pLZRS-BMN-IRES-GFP vector. The resulting vector is designated as LZRS-BMN-HA-1-IRES-GFP (HA-1 vector). The HA-1 vector was transfected into a Phoenix-A packaging cell line using the calcium phosphate method as described by the manufacturer (Life Technologies, Breda, The Netherlands). Transfected cells were cultured in Iscoves modified Dulbecco's medium (IMDM) with 10% fetal calf serum (FCS). At day 2, puromycin (2 mg/mL) was added to select the transfected packaging cells that expressed the puromycin resistance gene that was present in the retroviral vector. After 10 to 14 days, 6×10^6 puromycin-resistant cells were plated per 10-cm petri dish (Becton Dickinson, San Jose, CA) in 10 mL complete medium without puromycin. After 48 hours, the retroviral supernatant was harvested, centrifuged, and frozen in 1 mL aliquots at –70°C. The $HA\text{-}1^-$ Epstein-Barr virus–transformed B cell lines (EBV-LCLs) and HeLa cells transduced with HA-1 vector induced significant HA-1–specific proliferation and tumor necrosis factor (TNF)-α release and were lysed by HA-1–specific CTL clones demonstrating the proper expression of the HA-1 CTL epitope in transduced cells (data not shown).

Isolation of $CD34^+$ Dendritic Cell Progenitors, Retroviral Transduction, and DC Culture

Bone marrow aspirate or SC-mobilized blood was collected from donors who had given informed consent. $CD34^+$ cells were isolated using an immunomagnetic-bead cell-selection system (Clinimacs; Miltenyi Biotech, Bergisch Gladbach, Germany). The $CD34^+$ cells were cultured in IMDM and 10% autologous heparinized plasma at a concentration of 3×10^5 cells/mL in 24-well flat-bottomed tissue culture plates (Costar; Corning, Acton, MA) in the presence of a cytokine mix consisting of granulocyte-macrophage colony-stimulating factor (GM-CSF), 100 ng/mL (Genzyme, Leuven, Belgium); TNF-α, 2 ng/mL (Genzyme); and FLT3 ligand (FLT3-L), 100 ng/mL (R&D Systems, Minneapolis, MN). At day 2, the cells were transduced with retroviral supernatants with the use of recombinant human fibronectin fragments CH-296 (Retronectin; Takara, Otsu, Japan) as described elsewhere [22]. Twenty-four hours after transduction, the $CD34^+$ cells were transferred into tissue-culture plates and further cultured in IMDM supplemented with 10% autologous plasma and the cytokine mix. On day 4, this medium was refreshed with the addition of interleukin (IL)-4 (Genzyme), 500 U/mL. After 10 to 12 days, the retrovirally transduced DCs with HA-1 cDNA (HA-1–transduced DCs) were phenotypically analyzed and used as stimulator cells. The percentage of GFP^+ cells was measured at days 2 and 10 to determine the transduction efficiency and stability of the transgene.

Monoclonal Antibodies, $HA\text{-}1^{A2}$ Tetramers, and Phenotype Analysis

Fluoroscein isothiocyanate– or PE-conjugated monoclonal antibodies (MoAbs) against immunoglobulin (Ig)G1, IgG2, HLA-DR, CD1a, CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD20, CD80, and CD86 (Becton Dickinson) and CD83 (Immunotech, Marseille, France) were used at appropriate dilutions. The PE-conjugated $HA\text{-}1^{A2}$ tetramers were generated as described previously [23]. For the phenotypical analysis, the cells were surface labeled with MoAbs and/or with $HA\text{-}1^{A2}$ tetramers for 20 minutes at 37°C. The cells were then fixed with 1% paraformaldehyde and analyzed on a Becton Dickinson FACS-calibur flow cytometer. Data were analyzed using CellQuest (Becton Dickinson) and WinMDI (version 2.8 J. Trotter; Scripps Research Institute, La Jolla, CA) software.

HA-1 Peptide

The HA-1 peptide was synthesized using a semiautomatic multiple peptide synthesizer according to the reported sequence VLHDDLLEA [24]. The purity of the peptide was >95%, as analyzed by reverse-phase high-pressure liquid chromatography.

In Vitro Induction of HA-1–Specific CTLs from $HA\text{-}1^-$ SC Donors

Responder peripheral blood mononuclear cells (PBMCs) ($20\text{-}30 \times 10^6$ cells) from $HLA\text{-}A2^+$ $HA\text{-}1^-$ SC donors were stimulated with autologous HA-1–transduced DCs at a responder-to-stimulator ratio of 8:1 in 24-well tissue culture plates containing 1 mL/well IMDM, 10% autologous plasma, 6 IU/mL IL-2, 1U/mL IL-12 (R&D Systems). On

Table 1. *Retroviral Transduction Efficiency of CD34⁺ DC Precursor Cells**

Cytokines	Retroviral Transduction Day	% GFP⁺ Cells (Range) Day 6-7	Day 12-13
GMCSF⁺ TNF-α	1	11 (7-12)	10 (8-13)
	2	26 (22-31)	25 (20-31)†
	3	23 (21-24)	24 (20-26)
GMCSF⁺ TNF-α + FLT3-L	1	13	13
	2	37 (32-41)	32 (29-38)†
	3	24 (22-28)	26 (21-27)

*CD34⁺ cells derived either from bone marrow or from stem cell–mobilized peripheral blood were cultured with the indicated cytokines and transduced at the indicated days of culture. The GFP⁺ cells at day 6-7 and day 12-13 of the cultures were determined by FACS analysis.

†The statistical difference between the indicated values is significant ($P < .05$) in paired t test.

day 5, 60 IU/mL of IL-2 was added. On day 7, the CTLs were depleted of CD4⁺ T-cells and restimulated (first restimulation) with HA-1–transduced DCs at a responder-to-stimulator ratio of 8:1. After 24 hours and then every other 3 days, 120 IU/mL IL-2 was added. On day 14, the CTLs were restimulated (second restimulation) using (1) HA-1–transduced DCs (CTL#2, CTL#4), (2) HA-1 peptide–pulsed DCs (CTL#3), or (3) HA-1–transduced EBV-LCLs (CTL#1). From day 21 on, all CTLs were restimulated with peptide-pulsed (1 mg/mL) monocytes. The CTLs were analyzed with HA-1^{A2} tetramers and for HA-1–specific cytotoxic activities prior to each restimulation.

Target Cells, CTL Clones

Target Cells. EBV-LCLs were cultured in IMDM, 10% FCS. Phytohemagglutinin (PHA)-activated T-cell blasts (PHA blasts) were induced by stimulation of PBMCs with 0.5% PHA in RPMI, 15% human serum, and 120 IU/mL IL-2. On day 7, the PHA blasts were harvested and used as target cells.

CTL Clones. The mHag HA-1–specific CTL clone 3HA15 and the control mHag HA-3–specific CTL clone 5HO11 were previously isolated from the PBMCs of GVHD patients who received HA-1– or HA-3–mismatched SCT [3].

T-Cell Proliferation Assays

Responder T-cells ($1\text{-}2 \times 10^4$ cells/well) were cocultured with irradiated stimulator cells ($2\text{-}10 \times 10^4$ cells/well) in 96-well flat-bottomed microtiter plates for 72 hours in 200 μL of RPMI supplemented with 15% human serum. Sixteen hours before harvesting, 0.5 Ci of ^{3}H-thymidine was added. The ^{3}H-thymidine incorporation was determined by liquid scintillation counting. The results are expressed as the mean of triplicate cultures. The standard error of the mean (SEM) of the results did not exceed 15%.

Cell-Mediated Lympholysis Assay

^{51}Cr-labeled target cells (3000 cells/well) were incubated with serial dilutions of effector cells in 96-well round-bottomed microtiter plates (Costar 3799) at 37°C. After 4 hours, the cell-free supernatants were harvested for γ counting. The percent specific lysis was calculated as follows: % specific lysis = (experimental release – spontaneous release)/(maximal release – spontaneous release) × 100%.

Spontaneous release and maximal release are the chromium release of target cells in culture medium alone and in culture medium containing 1% triton-X 100, respectively. The SEM of the results did not exceeded 5%. If the SEM of the results exceeded 5%, the results were discarded.

Statistical Analysis

The effects of FLT-3 ligand on transduction efficiency, cell viability, and cell yields were tested using a paired t test. The Mann-Whitney test was used to test the significance of the difference in generation of HA-1–specific CTLs using HA-1–transduced DCS compared with that using peptide-pulsed DCs. The results are expressed as 2-tailed P values within 95% confidence intervals.

RESULTS

Retroviral Transduction of CD34⁺ Cells

Bone marrow or SC-mobilized blood-derived CD34⁺ cells, cultured with the different cytokine cocktails to induce DC differentiation, were transduced at day 1, 2, or 3 of culture (Table 1). Day 2 was the optimal day of transduction, with mean transduction rates of 26%. Addition of FLT-3 ligand in the cytokine cocktail significantly increased the mean transduction efficiency from 26% to 37% ($P < .05$) (Table 1). FLT-3 ligand also significantly increased the cell viability and cell yields ($P < .05$). After 12 to 13 days of DC culture, the total number of cells increased 10- to 15-fold. During this period, the percentage of GFP⁺ cells remained unchanged, demonstrating the stability of the transgene (Table 1). CD34⁺ cells derived from both bone marrow and SC-mobilized blood showed equal transduction efficiencies (data not shown).

Immunophenotype of Retrovirally Transduced DCs

Retrovirally transduced cells were analyzed for lineage and cell activation markers (Table 2, Figure 1A) at day 13 of DC culture. Neither GFP⁺ (transduced) nor GFP⁻ (untransduced) cells in the cultures expressed the cell lineage markers CD3, CD14, CD19, CD56, or CD66. HLA-DR was expressed on 83% (±16%) of the transduced, and on 78%

Table 2. *Immunophenotype of Retrovirally Transduced DCs**

Surface Molecule	% GFP⁺ Cells	% GFP⁻ Cells
HLA-DR	83 ± 16	78 ± 9
CD1a	74 ± 17	67 ± 6
$CD11c^{Bright}$	81 ± 12	74 ± 11
CD3	<1	<1
CD14	<1	<1
CD19	<1	<1
CD56	<1	<1
CD66	<1	<1

*Retrovirally transduced DCs were analyzed for the surface expression markers on day 13 of DC culture. Data are representative of 5 independent DC cultures.

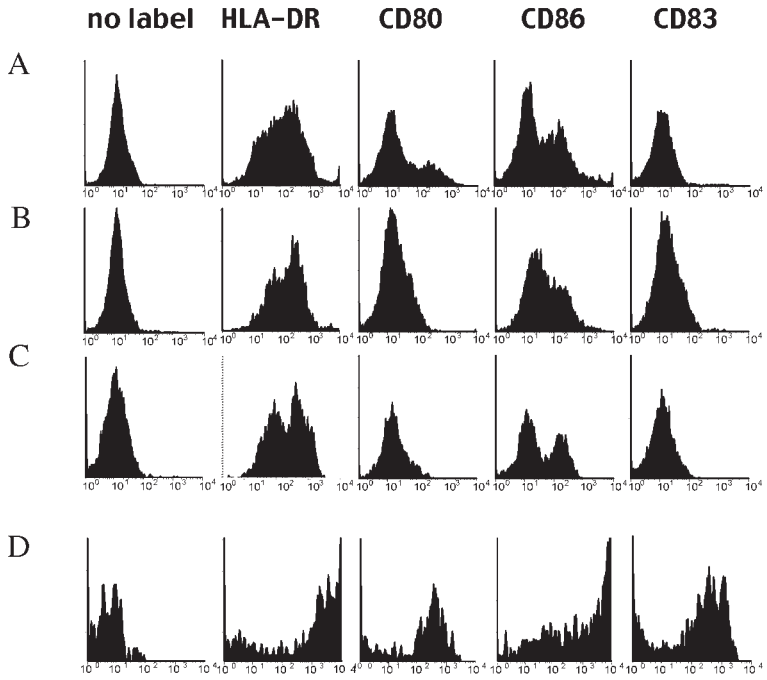

Figure 1. Immunophenotype of retrovirally transduced DCs. Retrovirally transduced DCs were stained with PE-conjugated MoAbs specific for HLA-DR, CD80, CD86, and CD83. Histograms represent the expression of markers on GFP$^+$ cells. Similar results were obtained by putting the gates on GFP$^+$, HLA-DR$^+$ CD14$^-$ cells. A, Retrovirally transduced DCs on day 12 of DC culture, prior to culturing with responder PBMCs. B, Retrovirally transduced DCs on day 2 of coculturing with responder PBMCs. C, Retrovirally transduced DCs on day 7 of coculturing with responder PBMCs. D, Retrovirally transduced DCs cocultured for 48 hours with CD40-L transfected fibroblasts.

(±9%) of the nontransduced cells, indicating that approximately 80% of both the transduced and the nontransduced cell fractions comprised HLA-DR–positive, lineage marker–negative DCs. Bright staining with the monocyte/DC marker CD11c showed on 81% of GFP$^+$ and 74% of GFP$^-$ cells. CD1a expression was similar on GFP$^+$ and GFP$^-$ cells, 67% and 74%, respectively. The expression levels of HLA-DR, CD80, and CD86 were moderate or low on both transduced (Figure 1A) and nontransduced DCs (data not shown). Furthermore, little or no expression of CD83 on either GFP$^+$ or GFP$^-$ cells indicated that both transduced and nontransduced DCs showed an immature DC phenotype (Figure 1A).

Long-term Persistence of Retrovirally Transduced DCs in the CTL Cultures

To estimate whether retrovirally transduced DCs can express the HA-1 vector for prolonged periods in the CTL cultures, the HA-1–transduced DCs were cocultured with autologous PBMCs and analyzed at different days of culture. The HA-1–transduced GFP$^+$ DCs were detected in the cultures for up to 7 days (Figures 1B and 1C). During this period, the transduced DCs retained their immature phenotype (Figure 1B and 1C). In control assays, retrovirally transduced DCs significantly up-regulated HLA-DR, CD80, and CD86 and induced CD83 after 48 hours of culture on a CD40-ligand–expressing fibroblast line (Figure 1D), indicating that the intrinsic maturation pathway of retrovirally transduced DCs was functional but was not triggered by coculture with autologous PBMCs.

Efficient Presentation of the mHag HA-1 CTL Epitope by HA-1–Transduced DCs

To estimate the antigen-presenting capacity, HA-1–transduced DCs were used to stimulate the proliferation of the HA-1–specific CTL clone 3HA15 (Figure 2). Nontransduced DCs pulsed with 1 μg/mL HA-1 peptide were used as control APCs. The HA-1–specific CTL clone 3HA15 was stimulated strongly by HA-1–transduced DCs and by peptide-pulsed DCs in a similar fashion. The mHag HA-3– specific CTL clone 5HO11, which was used as negative control, was

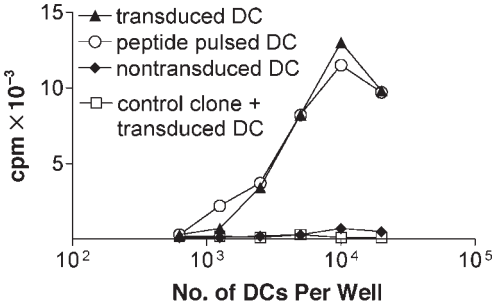

Figure 2. Presentation of the HA-1 CTL epitope by HA-1–transduced DCs. Serial dilutions of HA-1–transduced DCs with 25% transduction efficiency were used to stimulate the proliferation of the HA-1–specific CTL clone 3HA15 (▲) and the control HA-3–specific CTL clone 5HO11 (□). Peptide-pulsed (1 mg/mL) DCs (○) and nontransduced DCs (◆) were also used as control APCs.

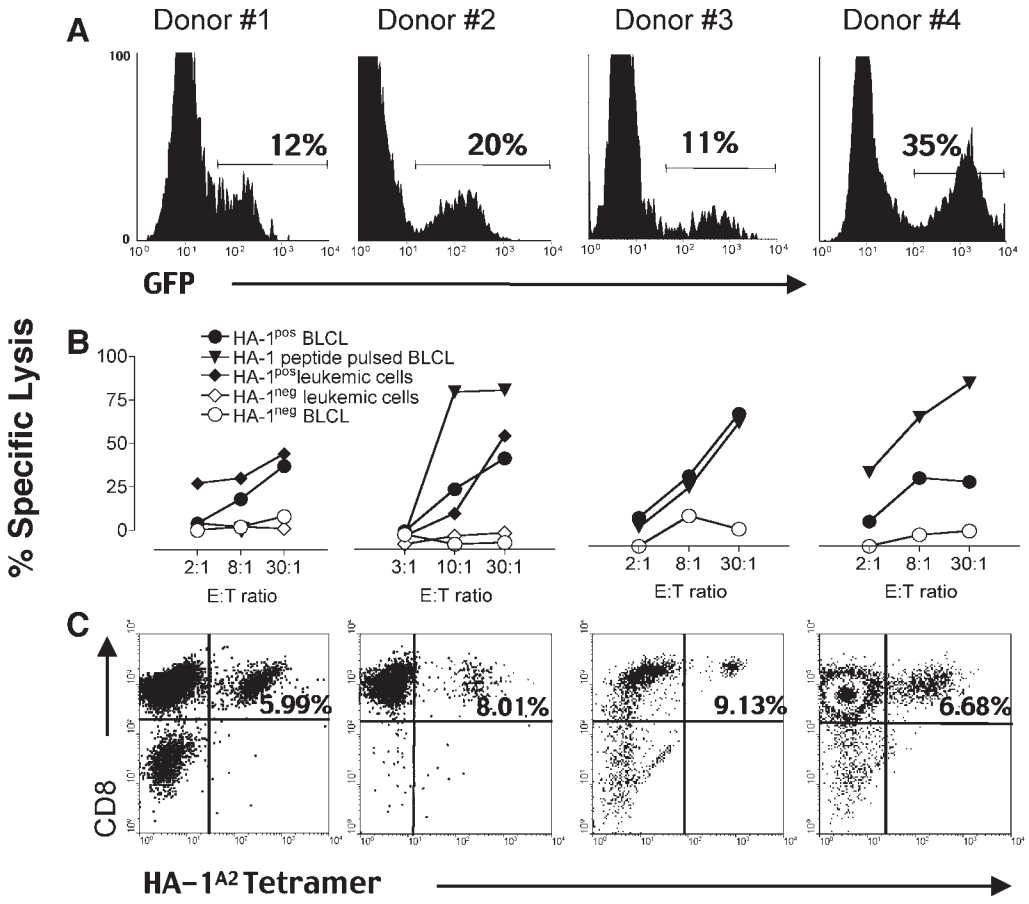

Figure 3. Induction of HA-1–specific CTLs by HA-1–transduced DCs. CD34$^+$ cells were isolated from bone marrow (donor #1) or from stem cell–mobilized peripheral blood (donors #2, #3, and #4) of HLA-A2$^+$ HA-1$^-$ donors. CD34$^+$ cells were transduced at day 2. A, The transduction efficiency and the GFP expression levels of DCs at day 12. The GFP$^+$ cells were negative for lineage markers and contained 78%, 82%, 87%, and 76% HLA-DR$^+$ DCs for donors #1, #2, #3, and #4, respectively. B, The cytotoxic activity of CTLs measured at day 21. C, Immunostaining of CTLs with CD8 antibodies plus HA-1^{A2} tetramers.

not stimulated at all, showing that retrovirally transduced DCs induce only specific T-cell proliferation.

Efficient Induction of HA-1–Specific CTLs by HA-1–Transduced DCs

HA-1–transduced DCs were used as stimulator cells to stimulate PBMCs from 6 HLA-A2$^+$, HA-1$^-$ healthy SC donors. The transduction efficiencies varied from 11% to 35% (Figure 3A). Independent of the variable transduction efficiencies, HA-1–specific CTLs were induced from 4 donors after 1 (donor #1) or 2 rounds (donors #2, #3, and #4) of restimulation with HA-1–transduced DCs. The CTLs showed high avidity for the HLA-A-2/HA-1 ligand as demonstrated by the significant lysis of HA-1 peptide-pulsed EBV-LCLs and EBV-LCLs and leukemic (acute lymphoblastic leukemia) cells that naturally express the HLA-A2/HA-1 ligand. Analysis with the HA-1^{A2} tetramers revealed that 6% to 9% of the lymphocytes in the cultures were HA-1–specific CTLs (Figures 3B and 3C).

From 2 other donors, CTLs could be generated neither with HA-1–transduced DCs nor with peptide-pulsed DCs (data not shown).

Comparison of HA-1–Transduced and Peptide-Pulsed DCs for the Induction of HA-1–Specific CTLs

In parallel with the HA-1–transduced DCs, peptide-pulsed DCs were used to generate HA-1–specific CTLs in donors #2 and #3 (Figure 4A). For the latter strategy, the DCs were pulsed with 1 μg/mL peptide for 2 hours in serum-free medium, conditions that had previously been found to be optimal [10].

In donor #2, HA-1–transduced DCs induced strong HA-1–specific cytotoxic activity within 21 days. The CTL culture contained 8% HA-1^{A2} tetramer$^+$ cells. In contrast, peptide-pulsed DCs induced a weak HA-1–specific CTL response, with only 2% HA-1^{A2} tetramer$^+$ cells at day 21. At day 28, after another round of stimulation, CTLs induced with peptide-pulsed DCs also showed significant HA-1–specific cytotoxic activity and contained 13% HA-1^{A2} tetramer$^+$ cells.

In donor #3, HA-1–transduced DCs induced strong HA-1–specific CTLs within 21 days. The culture contained 9% HA-1^{A2} tetramer$^+$ cells. Peptide-pulsed DCs, however, failed to induce an HA-1–specific CTL response at day 21. The CTL culture contained less than 1% HA-1^{A2} tetramer$^+$ cells. At day 28, the HA-1^{A2} tetramer$^+$ cells increased up to

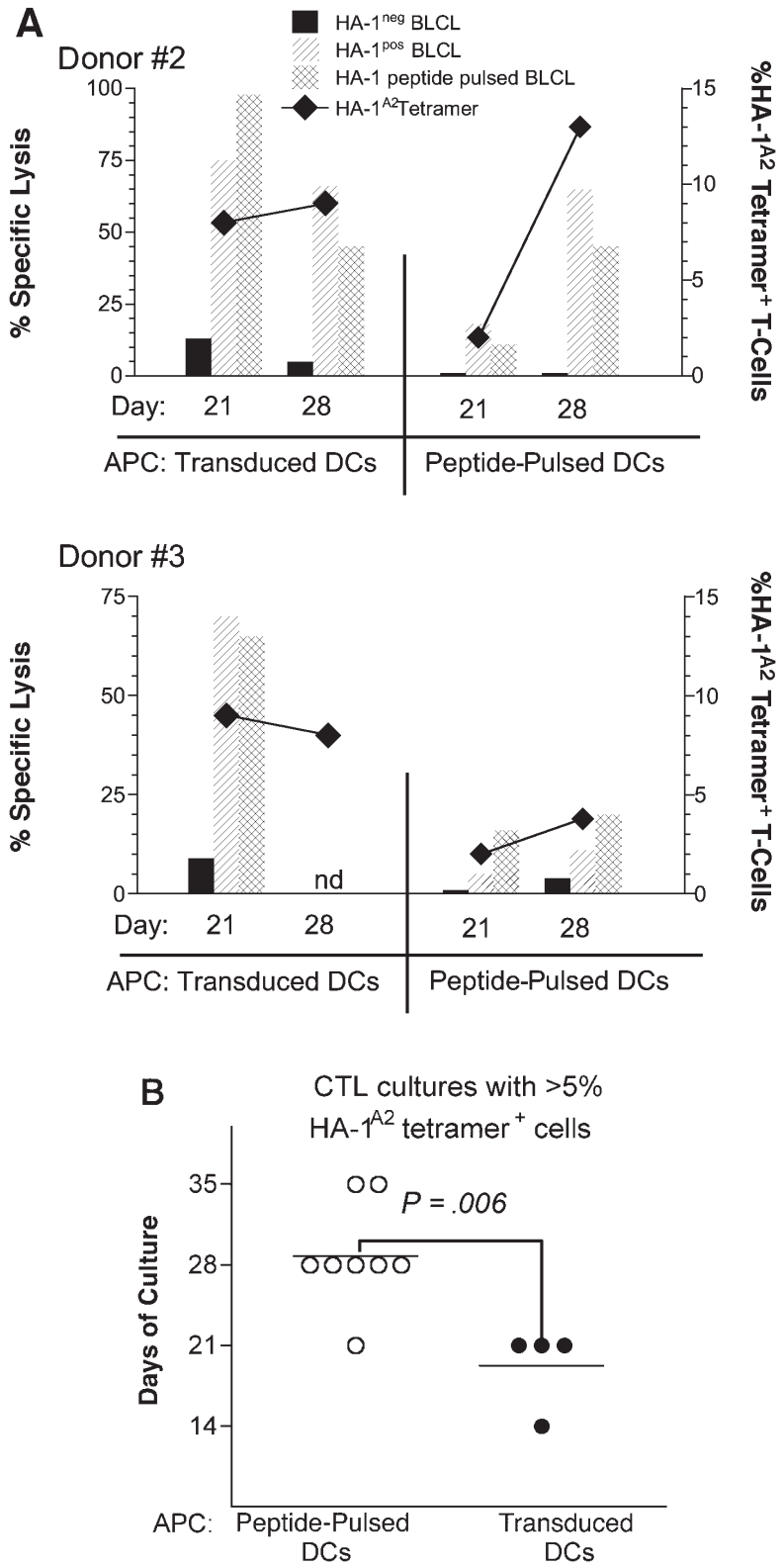

Figure 4. Comparison of HA-1–transduced and peptide-pulsed DCs for the induction of HA-1–specific CTLs. A, In donors #2 and #3 the HA-1–specific CTL cultures were simultaneously induced with retrovirally transduced DCs and with peptide-pulsed DCs. The percent specific lysis levels of the indicated target cells are presented on the left axes and the percentages of HA-1^{A2} tetramer$^+$ T-cells in the cultures are presented on the right axes. The percent lysis levels are shown only for the highest effector target ratio of 30:1. B, Comparison of HA-1–specific CTLs generated by peptide-pulsed and HA-1–transduced DCs from different individuals. For all CTLs, the day of culture during which HA-1^{A2} tetramer$^+$ cells reached 5% or more are shown.

4% but the HA-1–specific cytotoxic activity of the CTLs remained poor. Neither the cytotoxic activity nor the percentage of tetramer$^+$ cells in the peptide-induced CTL line could be further improved by repeated rounds of restimulation (data not shown).

In addition, we determined the day of culture on which the HA-1^{A2} tetramer$^+$ cells reached 5% or more in several other in vitro–induced HA-1–specific CTL lines. Comparison of the CTLs induced with HA-1–transduced DCs (n = 4) with CTLs induced with peptide (n = 8) revealed a significant difference in favor of HA-1–transduced DCs for the induction time of HA-1–specific CTLs (Figure 4B).

DISCUSSION

In this study we show that DCs that are transduced with the cDNA coding for the HA-1 CTL epitope are efficient APCs for the in vitro induction of mHag HA-1–specific CTLs from HA-1$^-$ SC donors. HA-1–transduced DCs trigger HA-1–specific CTL responses with more rapid kinetics than the CTLs induced with peptide-pulsed DCs.

In various model systems and in clinical studies, peptide-pulsed DCs were successfully used to induce tumor-specific T-cell responses [25-34]. Earlier, we showed the in vitro generation of mHag HA-1/HA-2–specific CTLs from unprimed SC donors with the use of DCs pulsed with synthetic HA-1/HA-2 peptides [9,10]. However, a major drawback of the peptide-pulsing strategy is the "peptide dose." Optimal induction and expansion of high-avidity CTLs with peptide-pulsed DCs requires a critical dose of peptide that needs to be determined carefully. It has been shown that peptide concentrations that are too high can induce low-avidity CTLs or may even deplete T-cell responses by induction of anergy or by activation-induced cell death [35,36]. On the other hand, peptide concentrations that are too low may not induce T-cell responses at all. In line with these observations, our previous studies revealed that HA-1 peptide optimally induces CTLs within a narrow window, with 1 μg/mL being the optimal dose. Fivefold higher or lower concentrations were detrimental [10]. Another drawback of peptide-pulsing strategy is the loss of peptides from the DC surface. Because the precursors of both CD4$^+$ and CD8$^+$ T-cells require sustained antigen presentation to become activated, it may be possible that synthetic peptides may dissociate from the major histocompatibility complex molecules before fully activating the unprimed T-cells. An advantage of introduction of tumor antigens in DCs is that the antigens can be continuously presented to T-cells. We therefore chose the retroviral gene delivery system because it readily transduces CD34$^+$ cells and allows stable expression of the transgene during the DC differentiation and CTL culture periods.

In our assays, HA-1–transduced DCs persisted in the in vitro CTL cultures for at least 7 days and induced HA-1–specific CTLs more rapidly than DCs pulsed with the optimum peptide dose. Thus, our results suggest that sustained presentation of HA-1 CTL epitope on HA-1–transduced DCs is an important factor that facilitates the in vitro generation of HA-1–specific CTLs.

Our results also show that strong HA-1–specific T-cell responses can be generated with relatively low transduction efficiencies. We established a maximum of 40% transduction efficiency, with a mean efficiency of 20%. However, we have observed that strong HA-1–specific CTLs can be generated even with 11% transduction efficiency. Thus, retrovirally transduced DCs efficiently present the CTL epitopes to unprimed T-cells. Therefore, high transduction efficiencies are not per se necessary for the induction of relevant CTL responses.

A possible advantage of expression of the target antigen in DCs may be the activation of antigen-specific CD4$^+$ T-helper cells [37]. However, we have as yet no evidence that the mHag HA-1 segment inserted in DCs contains an HLA class II–restricted T-cell epitope. The CD4$^+$ T-cells isolated from our in vitro cultures did not show HA-1 specificity (data not shown). The lack of specific or nonspecific CD4 help may hamper the efficient generation of HA-1–specific CTLs and may be the reason why we could not generate HA-1–specific CTLs from 2 SC donors using either peptide-pulsed or HA-1–transduced DCs.

In conclusion, our results show the rapid induction of HA-1 CTLs by HA-1–transduced DCs, probably due to sustained antigen presentation to unprimed CTL precursors. Retrovirally transduced DCs can thus function as adequate APCs for the in vitro induction of HA-1–specific CTLs for adoptive immunotherapy and for vaccination of the HA-1$^-$ SC donors or HA-1–positive SC recipients who undergo HA-1–mismatched SCT [38].

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